

Claim 27 (new) A method of producing ergosterol or one or more intermediate products of its biosynthesis, comprising,

a) designing a plasmid, into which the following genes are inserted:

- i) **t-HMG**, an HMG-Co-A-reductase gene,
 ERG9, a squalene synthetase gene,
 SAT1, an Acyl-CoA: sterol-acyl transferase gene, and
 ERG1, a squalene epoxidase gene,

or

- ii) **t-HMG**, an HMG-Co-A-reductase gene, and
 ERG9, a squalene synthetase gene,

or

- iii) **t-HMG**, an HMG-Co-A-reductase gene, and
 SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

- iv) **t-HMG**, an HMG-Co-A-reductase gene, and
 ERG1, a squalene epoxidase gene,

or

- v) **ERG9**, a squalene synthetase gene, and
 SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

- vi) **ERG9**, a squalene synthetase gene, and
 ERG1, a squalene epoxidase gene,

or

- vii) **SAT1**, an acyl-CoA: sterol-acyl transferase gene, and
 ERG1, a squalene epoxidase gene,

or

- viii) one of the genes selected from the group consisting of **ERG9**, **SAT1** and

ERG1,

b) transforming a microorganism with a plasmid mentioned in i) to vii), or,

simultaneously or in succession, with two or more of the plasmids mentioned in viii), and

- c) culturing the transformed microorganism under conditions in which it produces ergosterol and intermediate product of ergosterol biosynthesis.

Claim 28 (new) A method according to claim 27, wherein **ERG1**, a squalene epoxidase gene, is further inserted into the plasmid mentioned in ii), iii) or v); or **SAT1**, an acyl-CoA: sterol-acyl transferase gene, is further inserted into the plasmid mentioned in ii).

Claim 29 (new) The method according to claim 27, wherein the genes in each case with the plasmids are first introduced independently of one another into microorganisms of the same species.

Claim 30 (new) The method according to claim 27, wherein the intermediate product is squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol, or ergosta-5,7-dienol.

Claim 31 (new) The method according to claim 27, wherein the intermediate product is a sterol with a 5,7-diene structure.

Claim 32 (new) The method according to claim 27, wherein the plasmid is YEpH2, YDpUHK3 or pADL-SAT1.

Claim 33 (new) The method according to claim 27, wherein the microorganism is a yeast.

Claim 34 (new) The method according to claim 33, wherein the yeast is the species **S. cerevisiae**.

Claim 35 (**new**) The method according to claim 33, wherein the yeast is the strain **S. cerevisiae** AH22.

Claim 36 (**new**) A yeast strain **S. cerevisiae** AH22 comprising at least one gene selected from the group consisting of **t-HMG**, an HMG-Co-A-reductase gene, **ERG9**, a squalene synthetase gene; **SAT1**, an Acyl-CoA sterol-acyl transferase gene; and **ERG1**, a squalene epoxidase gene.

Claim 37 (**new**) The plasmid YEpH2, which comprises an **ADH**-promoter, a **t-HMG** gene, and a **TRP**-terminator, as shown in Fig. 1.

Claim 38 (**new**) The plasmid YDpUHK3, which comprises the **ADH**-promoter, the **t-HMG** gene, the **TRP**-terminator, the gene for kanamycin resistance and the **ura3** gene, as shown in Fig. 2.

Claim 39 (**new**) The plasmid pADL-SAT1, which comprises the **SAT1** gene and the **LEU2** gene of YEp13, as shown in Fig. 3.

Claim 40 (**new**) A method for producing ergosterol, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces ergosterol.

Claim 41 (**new**) A method for producing an intermediate product in the biosynthesis of ergosterol, which is squalene, farnesol, geraniol, lanosterol, zymosterol, 4,4-dimethylzymosterol, 4-methylzymosterol, ergost-7-enol, or ergosta-5,7-dienol, or a combination thereof, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces said intermediate product.

Claim 42 (**new**) A method for producing an intermediate sterol product with a 5,7-diene structure in the biosynthesis of ergosterol, comprising transforming a microorganism with a plasmid according to claim 37, and culturing the transformed microorganism under conditions in which it produces said intermediate sterol product.

Claim 43 (**new**) An expression cassette that comprises a **t-HMG** gene operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene operatively linked to an **ADH**-promoter and a **TRP**-terminator.

Claim 44 (**new**) An expression cassette that comprises a **t-HMG** gene operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **SAT1** gene operatively linked to an **ADH**-promoter and a **TRP**-terminator, and an **ERG9**-gene operatively linked to an **ADH**-promoter and a **TRP**-terminator.

Claim 45 (**new**) A combination of expression cassettes, which comprises

- a) a first expression cassette, on which an **ADH**-promoter, a **t-HMG**-gene, and a **TRP**-terminator are located,
- b) a second expression cassette, on which an **ADH**-promoter, a **SAT1**-gene and a **TRP**-terminator are located,

and

- c) a third expression cassette, on which an **ADP**-promoter, an **ERG9**-gene and a **TRP**-terminator are located.

Claim 46 (**new**) A method of producing a microorganism that can be used for producing ergosterol, comprising transforming a microorganism with an expression cassette according to claim 43.

Claim 47 (**new**) The method according to claim 46, wherein the microorganism is a yeast.

Claim 48 (**new**) A microorganism which comprises an expression cassette according to claim 43.

Claim 49 (**new**) The microorganism according to claim 48, which is a yeast.

Claim 50 (**new**) A method for producing ergosterol, comprising culturing a microorganism according to claim 48 under conditions in which it produces ergosterol.

Claim 51 (**new**) A method for producing one or more intermediate products in the biosynthesis of ergosterol, comprising culturing a microorganism according to claim 48 under conditions in which it produces said intermediate products.

Claim 52 (**new**) The method according to claim 27, further comprising,
d) after the culturing is complete, extracting the ergosterol and its intermediate products from the cells and analyzing them, and
e) purifying the thus obtained ergosterol and its intermediate products, using column chromatography.

Claim 53 (**new**) A method for producing ergosterol or one or more intermediate products of its biosynthesis, comprising expressing in a microorganism a plasmid which comprises the following genes:

- i) t-**HMG**, an HMG-Co-A-reductase gene,
 ERG9, a squalene synthetase gene,
 SAT1, an Acyl-CoA: sterol-acyl transferase gene, and
 ERG1, a squalene epoxidase gene,

or

- ii) t-**HMG**, an HMG-Co-A-reductase gene, and
 ERG9, a squalene synthetase gene,

or

- iii) **t-HMG**, an HMG-Co-A-reductase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

- iv) **t-HMG**, an HMG-Co-A-reductase gene, and
ERG1, a squalene epoxidase gene,

or

- v) **ERG9**, a squalene synthetase gene, and
SAT1, an acyl-CoA: sterol-acyl transferase gene,

or

- vi) **ERG9**, a squalene synthetase gene, and
ERG1, a squalene epoxidase gene,

or

- vii) **SAT1**, an acyl-CoA: sterol-acyl transferase gene, and
ERG1, a squalene epoxidase gene,

or

- viii) one of the genes selected from the group consisting of **ERG9**, **SAT1** and **ERG1**, and isolating the expressed ergosterol or intermediate products of its biosynthesis.